CLAIMS

We claim:

1. A composition comprising:

a) a first ligation probe comprising a 5'-iodide moiety; and

b) a second ligation comprising a 3'-sulfur moiety;

wherein at least one of said ligation probes comprises at least a first electron transfer moiety with a first redox potential.

2. A composition according to claim 1 further comprising a target nucleic acid strand with a first domain that hybridizes to said first ligation probe and a second domain that hybridizes to said second ligation probe.

3. A composition according to claim 1 further comprising a third ligation probe comprising a second ETM with a second redox potential different from said first redox potential.

4. A composition according to claim 1 further comprising a fourth ligation probe comprising a third ETM with a third redox potential different from said first or said second redox potential.

5. A composition according to claim 1 further comprising a fifth ligation probe comprising a fourth ETM with a fourth redox potential different from said first, said second or said third redox potential.

6. A composition according to claim 3 wherein said first ligation probe comprises an interrogation position with a first base and said third ligation probe comprises an interrogation position with a second base.

7. A composition according to 5 wherein said first ligation probe comprises an interrogation position with a first base, said third ligation probe comprises an interrogation position with a second base, said fourth ligation probe comprises an interrogation position with a third base, and said fifth ligation probe comprises an interrogation position with a fourth base.

8. A composition according to claim 7 wherein said first, third, fourth and fifth ligation probe comprise the 5' iodide moiety.

9. A composition according to claim 7 wherein said first, third, fourth and fifth ligation probe comprise the 3' sulfur moiety.

10. A method of chemical ligation, said method comprising:

a) providing a ligation substrate comprising:

i) a target nucleic acid strand;

ii) a first ligation probe comprising a 5'-iodide moiety; and

iii) a second ligation probe comprising a 3' sulfur moiety;

wherein at least one of said first and said second ligation probes comprises at least a first electron transfer moiety with a first redox potential;

b) ligating said first and said second ligation probes under conditions wherein said 5'-iodide moiety on said first ligation probe is displaced by said 3'-sulfur moiety on said second ligation probe to form a covalent linkage.

11. A method of detecting a target sequence in a sample comprising:

a) providing a ligation substrate comprising:

i) a target sequence comprising a first domain and an adjacent second domain;

ii) a first ligation probe that is hybridized to said first domain, wherein said first ligation probe comprises a 5'-iodide moiety;

iii) a second ligation probe that is hybridized to said second domain, wherein said second ligation probe comprises a 3'-sulfur moiety;

wherein at least one of said first and second ligation probes comprise at least one electron transfer moiety (ETM);

b) forming a ligation complex comprising said target sequence and a ligated strand by ligating said first and said second ligation probes under conditions wherein said 5'-iodide moiety on said first ligation probe is displaced by said 3'-sulfur moiety on said second ligation probe to form a ligated strand;

c) denaturing said ligation complex;

d) hybridizing said ligated strand to a capture probe on an electrode; and

e) detecting said electron transfer moiety as an indication of the presence of said target sequence in said sample.

12. A method according to claim 11 wherein said first ligation probe comprises said 3'-sulfur moiety and said second ligation probe comprises said 5'-iodide moiety.

13. A method according to claim 11 wherein said ETM is a ferrocene.

14. A method according to claim 11 wherein at least one of said ligation probes comprises a recruitment linker comprising two or more ETMs.

15. A method according to claim 11 wherein said electrode further comprises a self-assembled monolayer.

16. A method of detecting a target sequence in a sample comprising:

a) providing a ligation substrate comprising:

i) a target sequence comprising a first domain and an adjacent second domain;

ii) an electrode comprising a first ligation probe covalently attached to said electrode via an attachment linker, wherein said first ligation probe is hybridized to said first domain of said target sequence; and,

iii) a second ligation probe comprising at least one ETM and wherein said second ligation probe is hybridized to said second domain of said target sequence;

wherein said first ligation probe comprises a 3'-sulfur moiety and said second ligation probe comprises a 5'-iodide moiety;

b) forming a ligation complex comprising said target sequence and a ligated strand by ligating said first and said second ligation probes under conditions wherein said 5'-iodide moiety on said first ligation probe is displaced by said 3'-sulfur moiety on said second ligation probe to form a ligated strand;

c) denaturing said ligation complex, such that said ligated strand comprising said first and said second ligation probes is attached to said electrode ; and

d) detecting said electron transfer moiety as an indication of the presence of said target sequence in said sample.

17. A method of detecting a target sequence in a sample comprising:

a) providing a ligation substrate comprising:

i) a target sequence comprising a first domain and an adjacent second domain;

ii) an electrode comprising a first ligation probe covalently attached to said electrode via an attachment linker, wherein said first ligation probe is hybridized to said first domain of said target sequence; and,

iii) a second ligation probe hybridized to said second domain of said target sequence;

wherein said first ligation probe comprises a 3'-sulfur moiety and said second ligation probe comprises a 5'-iodide moiety;

b) forming a ligation complex comprising said target sequence and a ligated strand by ligating said first and said second ligation probes under conditions wherein said 5'-iodide moiety on said first ligation probe is displaced by said 3'-sulfur moiety on said second ligation probe to form a ligated strand;

c) denaturing said ligation complex, such that said ligated strand comprising said first and said second ligation probe is covalently attached to said electrode via said attachment linker;

d) hybridizing said ligated strand to a label probe comprising at least one ETM; and

e) detecting said electron transfer moiety as an indication of the presence of said target sequence in said sample.

18. A method according to claim 16 or 17 wherein said first ligation probe comprises said 5'-idodide moiety and said second ligation probe comprises said 3'-sulfur moiety moiety.

19. A method according to claim 16 or 17 wherein said ETM is a ferrocene.

20. A method according to claim 17 wherein said label probe comprises a recruitment linker comprising two or more ETMs.

21. A method according to claim 16 or 17 wherein said electrode further comprises a self-assembled monolayer.

22. A method of detecting a target sequence in a sample comprising:

a) providing a substrate comprising an array of electrodes each comprising:

i) a self-assembled monolayer (SAM); and,

ii) a different first ligation probe, wherein each of said first ligation probes is capable of hybridizing to a first domain of a target sequence:

iii) a plurality of target sequences comprising a first domain and an adjacent second domain;

iv) a plurality of second ligation probes capable of hybridizing to said second domain of said target sequences;

wherein said first ligation probes comprise a 3'-sulfur moiety and said second ligation probes comprise a 5'-iodide moiety;

b) forming ligation complexes comprising said target sequences and a plurality of ligated strands by ligating said first and said second ligation probes under conditions wherein said 5'-iodide moiety on said first ligation probes displaces said 3'-sulfur moiety on said second ligation probes to form a plurality of ligated strands;

c) denaturing said ligation complexes, such that said ligated strands are covalently attached to said electrodes;

d) hybridizing said ligated strands to a plurality of label probes comprising at least one ETM; and

e)detecting said electron transfer moiety as an indication of the presence of said target sequences in said sample.

23. A method for ligating two probes comprising:

a) providing a ligation substrate comprising:

i) a target sequence comprising a first domain and an adjacent second domain;

ii) a first ligation probe comprising a 3' OH moiety;

iii) a second ligation probe comprising a 5' OH moiety;

wherein at least one of said ligation probes comprises at least one covalently attached electron transfer moiety;

b) contacting said ligation substrate with a ligation chemical to ligate said ligation probes to form a ligated strand.

24. A method according to claim 23 wherein said ligation chemical is N-cyanoimidazole.

25. A method according to claim 23 wherein said ligation chemical is 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC).

26. A method for ligating two probes comprising:

a) providing a ligation substrate comprising:

i) a target sequence comprising a first domain and an adjacent second domain;

ii) a first ligation probe comprising a 3' phosphate moiety;

iii) a second ligation probe comprising a 5' amino moiety;

wherein at least one of said ligation probes comprises at least one covalently attached electron transfer moiety;

b) contacting said ligation substrate with a ligation chemical to ligate said ligation probes to form a ligated strand.

27. A method according to claim 26 wherein said ligation chemical is N-cyanoimidazole.

28. A method according to claim 26 wherein said ligation chemical is 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC).

29. A method for determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, said method comprising:

a) hybridizing a first ligation probe to said first domain, wherein said first ligation probe comprises:

i) a first base at an interrogation position;

ii) a 3'-sulfur moiety; and

iii) a first electron transfer moiety comprising a first redox potential;

b) hybridizing a second ligation probe to said second domain, wherein said second probe comprises a 5'-iodide moiety;

wherein if said first base of said first ligation probe is perfectly complementary to said detection position a ligation substrate is formed;

c) providing conditions wherein said 3'-sulfur moiety on said first ligation probe displaces said 5'-iodide moiety on said second ligation probe to form a ligated strand;

d) forming an assay complex comprising said ligated strand and a capture probe covalently attached to an electrode;

e) detecting the presence or absence of said ETM as an indication of the formation of said ligated strand; and

f) identifying the base at said detection position.

30. A method for determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain and a second target domain adjacent to said first domain and comprising said detection position, said method comprising:

a) hybridizing a first ligation probe to said first domain, wherein said first ligation probe comprises:

i) a first base at an interrogation position;

ii) a 5'-iodide moiety; and

iii) a first electron transfer moiety comprising a first redox potential;

b) hybridizing a second ligation probe to said second domain, wherein said second probe comprises a 3'-sulfur moiety;

wherein if said first base of said first ligation probe is perfectly complementary to said detection position a ligation substrate is formed;

c) providing conditions wherein said 3'-sulfur moiety on said second ligation probe displaces said 5'-iodide moiety on said first ligation probe to form a ligated strand;

d) forming an assay complex comprising said ligated strand and a capture probe covalently attached to an electrode;

e) detecting the presence or absence of said ETM as an indication of the formation of said ligated strand; and

f) identifying the base at said detection position.